VERIFICATION FOR TRANSLATION

I, Norio SAEKI, a national of Japan, c/o SAEKI & PARTNERS of 9th Floor, Taka-ai Building, 15-2, Nihonbashi 3-chome, Chuo-ku, Tokyo 103-0027, Japan, do hereby solemnly and sincerely declare:

1) THAT I am well acquainted with the Japanese language, English language, and

2) THAT the attached is a true, accurated and faithful translation into the English language made by me of Japanese Patent Application No. 10-333284/1998 filed to the Japanese Patent Office on November 24, 1998.

Signed this 7th day of October , 2004 .

Norio SAEKI

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[Title of the Invention] Nucleic Acid Capable of Specifically Binding to Raf-1

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[Name of Document] Abstract 1

[Necessity for Proof] necessary

[Document] Specification

[Title of the Invention] NUCLEIC ACID CAPABLE OF BINDING SPECIFICALLY TO RAF-1

[Claims]

[Claim 1]

A nucleic acid capable of specifically bound to Raf-1 protein wherein nucleic acid containing any one of base sequences of sequence Nos. 1 to 4 of Sequence Listing or a base sequence in which at least one base thereof is deleted and substituted with another base and/or at least one base is added or substituted, or base sequence comprising complementary strand thereof.

[Claim 2] The nucleic acid as claimed in claim 1, which nucleic acid is an RNA.

[Claim 3] An agent for controlling cell signal transduction which agent is made of the nucleic acid as claimed in claims 1 or 2.

[Claim 4] The controlling agent as claimed in claim 3, wherein the nucleic acid is an RNA.

[Claim 5] A method of controlling cell signal transduction using the nucleic acid as claimed in claims 1 or 2.

[Claim 6] The method as claimed in claim 5, wherein the nucleic acid is an RNA.

[Claim 7] A pharmaceutical composition containing the nucleic acid as claimed in claims 1 or 2.

[Claim 8] The pharmaceutical composition as claimed in

claim 7, which composition is used for treating cancers or inflammatory diseases.

[Claim 9] A method of selecting an RNA having specific binding ability to Raf-1, which comprises selecting the RNA having specific binding ability to Raf-1 from an RNA pool having random base sequences.

[Claim 10]The method as claimed in claim 9, wherein the RNA of the RNA pool having random base sequences is an RNA comprising 20 to 300 bases.

[Detailed Description of Invention]

[0001]

[Field of the Invention]

The present invention relates to a novel nucleic acid (aptamer) which is specifically bound to target proteins of Ras. More preferably, the present invention relates to a novel RNA aptamer which is specifically bound to Raf-1. Further, the present invention relates to control of signal transduction that induces proliferation or differentiation of cells using the nucleic acid of the present invention, and to a pharmaceutical composition using the same.

[0002]

[Related Art]

Ras is a guanine nucleotide binding protein, and is a protein which participates in signal transduction of cells. When a receptor of cells is activated, GDP binds to Ras protein in cells

and becomes "GDP binding Ras". "GDP binding Ras" is further phosphorylated which becomes "GTP binding Ras".

This "GTP binding Ras" is bound to target proteins of Ras such as Raf-1, B-Raf, RGL, Ral GDS, MEKK, P13K and the like, and transmit necessary signals into cells.

[0003]

These "target proteins of Ras" have a Ras binding domain to which the GTP binding Ras can be bound, and the GTP binding Ras is bound to this domain to transmit necessary signals into cells.

Ras is a key protein of intracellular signal transduction, and the "target proteins of Ras", such as Raf-1, are a center of the intracellular signal transduction system in which signals from Ras are transmitted according to the types.

[0004]

Accordingly, a substance capable of specifically blocking the binding domain with the GTP binding Ras in the "target proteins of Ras", if any, can specifically inhibit an intracellular signal transduction system by Ras, and it is useful to treat or prevent various diseases triggered by the signal transduction. For example, with respect to cancer cells, proliferation or differentiation of cancer cells can be inhibited by specifically controling the signal transduction that induces proliferation or differentiation with the "target cells of Ras" to treat cancers or inhibit metastasis.

[0005]

By the way, Raf-1, one of the "target cells of Ras" is a serine/threonine protein kinase present in a cytoplasm, and the activity is induced by binding to the GTP binding Ras. The activated Raf-1 activates MEK (MAPK/ERK kinase) which is one of the family of mitogen-activated protein kinases, transforming it into phosphorylates extracellular signal-regulated kinase, and thus participates in the signal transduction system (Daum, G., et al., (1994) Trends Biochem. Sci. 19, 474 - 480; Avruch, J., et al., (1994) Trends Biochem. Sci. 19, 279 - 283).

[0006]

In order to elucidate such an intracellular signal transduction system of Raf-1, a method of selectively inhibiting the function of Ras or Raf-1 has been utilized ((deVries-Smits, A. M., et al., (1992) Nature 357, 602 - 604)). These studies include inhibition of the Ras function with a Raf-1 mutant which is free from a kinase activity (Kolch, W., et al., (1991) Nature 349, 426 - 428), inhibition of a Raf-1 kinase with an antibody bound to a kinase domain of Raf-1 (Kolch, W., et al., (1966) Oncogene 13, 1305 - 1314) and the like.

[0007]

However, these inhibitors do not specifically inhibit a specific part of a signal transduction system with Ras or Raf-1, but inhibit many functions such as a function of binding to Ras, a kinase function and the like simultaneously and diversely.

Accordingly, a signal transduction system to be inhibited cannot be specified. Thus, individual specific mechanisms of a signal transduction system could not be clarified satisfactorily.

Consequently, the development of a molecular species capable of specifically inhibiting the binding of Ras to Raf-1 has become important for clarifying the role of the signal transduction system.

[0008]

At present, a downstream signaling pathway of Ras has not been completely clarified. When such a molecular species is developed, it is possible to elucidate the signaling pathway in which Ras participates using a molecular species capable of specifically inhibiting some specific routes and clarify the signaling pathway with target proteins of Ras in detail. In addition, it is possible to control the intracellular signal transduction. Consequently, various diseases in which the intracellular signal transduction participates, such as tumors and the like, can be treated and prevented.

[0009]

Meanwhile, the structural analysis of the "target proteins of Ras" in the intracellular signaling pathway in which Ras participates has been conducted. It has been known that the Ras binding domain (RBD) of Raf-1 is located from 51 to 131 residues in the N-terminus of Raf-1 (Vojtek, A. B., et al., (1993) Cell 74, 205 - 214; Chuang, E., et al., (1994) Mol. Cell. Biol. 14,

5318 - 5325).

[0010]

Further, nucleic acid molecular species (aptamer), such as RNA, DNA and the like, having high affinity for a certain target such as proteins, have been isolated by "in vitro selection" methods (Ellington, A. D. et al., (1990) Nature 346, 818 - 822; Tuerk, C. et al., (1990) Science 249, 505 - 510) (Bock, L. C., et al., (1992) Nature 355, 564 - 566; Qiu Qiu, Y. L., et al., (1994) Nucleic Acids Res. 22, 5229 - 5234; Gal. S. W., et al., (1998) Eur. J. Biochem. 252, 553 - 562; Bell, S. D., et al., (1998) J. Biol. Chem. 273, 14309 - 14314).

Additionally, present inventors have filed a patent application which relates to nucleic acid, especially aptamer, which is specifically bound to target proteins of Ras (Japanese patent application No.10-242596/1998).

[0011]

[Problems to be Resolved by the Invention]

The present invention is to provide nucleic acid molecular species which can specifically inhibit the binding to "GTP binding Ras" by being specifically bound to a Ras binding domain (RBD) of target proteins of Ras such as Raf-1, B-Raf, RGL, Ral GDS, MEKK, P13K and the like.

[0012]

The present inventors have found that a nucleic acid molecular species specifically bound to the Ras binding domain

of the "target proteins of Ras" can be obtained by using the in vitro selection method. For example, it has been possible to obtain a novel RNA aptamer targeting the Ras binding domain (RBD) of Raf-1, one of the "target proteins of Ras" by this method and to determine the RNA sequence thereof. This RNA aptamer can specifically inhibit the binding between Ras and Raf-1.

[0013]

In order to clarify the signaling pathway in which the target proteins of Ras participate and the physiological activity provided by the inhibition of the signal transduction, the development of substances which specifically inhibit the binding to Ras particularly having an important role in cells along with Raf-1, by being specifically bound to the target proteins of Ras, in more detail, Raf-1 and which have a strong activity have been in demand. Accordingly, the present invention is to provide an RNA aptamer that bound to a target protein of Ras, preferably Raf-1, especially its RBD, an agent for controlling a signal transduction system using this RNA aptamer, a method of controlling the same, and a pharmaceutical composition containing the same.

[0014]

[Means of Solving Problems]

The present invention is to provide a novel nucleic acid which specifically controls the intracellular signal transduction participated by Ras proteins and target proteins

of Ras.

The present invention relates to a novel nucleic acid, preferably RNA, capable of being specifically bound to the target proteins of Ras. More specifically, the present invention relates to a nucleic acid, preferably RNA, which is capable of being specifically bound to Raf-1.

[0015]

To be more precise, the present invention relates a nucleic acid capable of being specifically bound to Raf-1 wherein nucleic acid, preferably RNA, containing any one of base sequences of sequence Nos. 1 to 4 of Sequence Listing or a base sequence in which at least one base thereof is deleted, added or substituted. Or it relates to a nucleic acid, preferably RNA, having corresponding base sequence.

The RNA of the present invention shown in the above-mentioned sequence numbers have an binding ability to the Raf-1, and more specifically, the RNA is characterized in that they are specifically bound to the Ras binding domain (RBD) of Raf-1.

[0016]

An RNA of the present invention can also be reversely transcribed, as required, into single or double stranded DNA having complementary base sequences to the RNA. Accordingly, the present invention relates to nucleic acids such as RNAs, DNAs and the like, containing any one of base sequences of sequence

Nos. 1 to 4 of Sequence Listing or a base sequence in which at least one base thereof is deleted and substituted with another base and/or at least one base is added.

[0017]

Further, the present invention relates to agent for controlling cell signal transduction which agent is made of above-mentioned RNA, or the method for controlling cell signal transduction using said RNA.

Furthermore, the present invention relates to a pharmaceutical composition containing the above-mentioned RNA, more specifically it relates to a pharmaceutical composition for diseases in which the cell signal transduction participates such as treatment or prevention of cancer or inflammatory diseases.

[0018]

The "target proteins of Ras" of the present invention refers to Ras proteins which participate in the cell signal transduction, preferably a group of proteins forming an intracellular signal transduction system by interacting with GTP binding Ras proteins. Examples of the "target proteins of Ras" of the present invention include Raf-1, B-Raf, RGL, Ral GDS, MEKK, P13K and the like, however, this invention is not limited within the target. The "target proteins of Ras" of the present invention are preferably Raf-1 and the like.

[0019]

The present invention has clarified that nucleic acid molecular species capable of being specifically bound to the foregoing "target proteins of Ras" exist. Accordingly, the nucleic acid specifically bound to the "target proteins of Ras" in the present invention may be an RNA or a DNA. The RNA or the DNA is not particularly limited so long as it is specifically bound to the "target proteins of Ras". Further, the nucleic acid of the present invention may be specifically bound to only one "target protein of Ras" or to two or more "target proteins of Ras".

[0020]

The size of bases of the nucleic acid molecular species of the present invention is not particularly limited so long as it is sufficient to allow the specific binding to the "target proteins of Ras". It is between 20 and 300 bases, preferably between 20 and 150 bases, more preferably between 30 and 150 bases, further preferably between 30 and 120 bases. In the case of emphasizing the binding specificity, the longer size is preferable, however, in view of the availability such as by synthesis method or the like, the shorter size is preferable.

[0021]

The "aptamer" in the present invention refers to a nucleic acid molecular species capable of being bound to a specific domain of a protein, and the nucleic acid may be an RNA or a DNA. An aptamer made of an RNA is called an "RNA aptamer".

[0022]

The nucleic acid (aptamer) of the present invention can be produced by various methods. When the base sequence of the aptamer is known, it can be synthesized.

[0023]

When the base sequence of the aptamer of the present invention is unknown, the aptamer can be produced through selection by the known "in vitro selection" method (Ellington, A. D. et al., (1990) Nature 346, 818 - 822; Tuerk, C. et al., (1990) Science 249, 505 - 510).

Next, the "in vitro selection" method in the present invention is described.

[0024]

First, RNAs containing a random base sequence of 20 to 300 bases, preferably 30 to 100 bases, more preferably 30 to 70 bases are prepared. These RNAs are prepared by transcription from synthetic DNAs containing a random sequence.

A base sequence which is to be a primer in the PCR method is added to the 5'-terminus and the 3'-terminus of the DNAs. In this case, the primer is not particularly limited, however, a primer having a sequence of cleavage with a restriction endonuclease so as to be able to cleave this primer portion later is preferable. A size of the primer portion is not particularly limited. It is approximately between 20 and 50 bases, preferably between 20 and 30 bases. Further, a promoter sequence of a T7

RNA polymerase is added to the primer at the 5'-terminus, enabling the transcription reaction from DNA to RNA.

[0025]

In this manner, the RNA group (RNA pool) having the base sequences as the primer at both termini and the random base sequence in the center is prepared by transcription of the DNA.

Subsequently, the RNA in this RNA pool and the "target protein of Ras", for example, Raf-1 or a peptide comprising its binding domain are contacted to separate the RNA bound to the "target protein of Ras". The selected RNA is converted to a cDNA through reverse transcription, and it is amplified by PCR using the primers. The DNA amplified is transcribed into an RNA, and this is returned to the RNA pool.

[0026]

One cycle, termed a "round", comprises binding with the "target protein" of Ras in the RNA pool, the separation of the bound RNA, reverse transcription, amplification by PCR and transcription of the DNA. That is, one round means that the foregoing round is conducted once.

[0027]

When the foregoing round using the RNA pool is conducted, the amount of the RNA bound to the "target protein of Ras" in the RNA pool is increased, and further the amount of the RNA having the specific binding base sequence is increased, so that the RNA to be specifically bound can be selected by repeating

the round.

Such a round is conducted 5 to 50 times, preferably 5 to 30 times.

[0028]

The RNA sequences selected by the "in vitro selection" method as described above are determined by a usual method, and this RNA can also be converted to a cDNA through reverse transcription by a usual method. Further, the primer regions can be cleaved as required.

In this manner, the aptamers of the present invention can be obtained.

[0029]

The "in vitro selection" method of the present invention is described in more detail.

[0030]

(1) Preparing DNA pool:

Single-stranded DNA pool was obtained by synthesizing a single-stranded DNA pool (200 pmols, 1.2×10^{14} molecules) having a sequence of 5'-ggtaa tacga ctcac tatag ggagt ggagg aattc atcga ggcat-3' at the 5'-terminus and 5'-catat gcctt agcga cagca agctt ctgc-3' at the 3'-terminus and containing random 45 bases thereof. This single-stranded DNA pool was converted to a double-stranded DNA pool by PCR.

(2) Preparing RNA pool by transcription of DNA to RNA:

DNA pool obtained by (1) was converted to RNA pool containing

random sequence by in vitro transcription.

(3) Selecting RNA which bind to Raf-1RBD:

Selecting RNA capable of being bound to the Raf-1RBD which exist in the RNA pool obtained by (2) or (5).

[0031]

(4) Reverse transcription of the selected RNA to DNA:

DNA obtained by reverse transcription of RNA selected in (3) was amplified by PCR and made into DNA pool.

(5) Preparing RNA pool:

Amplified DNA pool obtained by (4) was transcribed in vitro, converting to RNA pool with increased ratio of RNA bound to Raf-1.

(6) Selecting RNA binding to Raf-1RBD, reverse transcription of RNA to DNA, amplification, repeating the transcription of DNA to RNA:

By repeating the process of (3) to (5) (round), ratio of RNA bound to Raf-1RBD which exist in the RNA pool increases.

(7) Determination of the RNA sequence which bind to Raf-1RBD:

RNA binding to Raf-1RBD obtained by (6) after repetition was reverse transcribed to obtain DNA. The obtained DNA is then introduced to E.Coli plasmid, cloning, and the DNA sequence is determined. Hereby, sequence of RNA bound to Raf-1RBD obtained by (6) is able to be determined.

Further, it is possible to obtain RNA which the 3'-terminus side is shortened by reverse transcription of RNA binding to Raf-1 RBD to DNA and then by obtaining DNA which 3'-terminus

side is shortened by PCR and transcribing it.

[0032]

Novel RNA which bind to Raf-1 RBD obtained in this manner is as described below and shown in sequence Nos. 1 to 4 of Sequence Listing.

[0033]

Sequence 1

gggaguggag gaauucaucg aggcauaugu cgacuccguc uuccuucaaa ccaguuauaa 60

auugguuuua gcauaugccu uagcgacagc aagcuucugc 100

[0034]

Sequence 2

gggaguggag gaauucaucg aggcaugacc ucccguggca guagggguaa aaauuaucuu 60

ccuacacuuc ucaugccuua gcgacagcaa gcuucugc 98

[0035]

Sequence 3

gggaguggag gaauucaucg aggcauaugu cgacuccguc uuccuucaaa ccaguuauaa 60

auugguuuua gcauaugccu uagcgacagc 90

[0036]

Sequence 4

gggaguggag gaauucaucg aggcauaugu cgacuccguc uuccuucaaa ccaguuauaa 60

auugguuuua gcauaugccu 80

[0037]

The Kd values of the RNAs shown in sequence Nos. 1, 2 and 4 and GST-RBD were as follows.

RNA of sequence 1: 124 nM

RNA of sequence 2: 295 nM

RNA of sequence 4: 176 nM

And, the RNAs of sequences 1 to 4 all inhibited the binding between Ras and Raf-1 RBD depending on the amounts.

[0038]

From these results, it is presumed that the RNAs of 99 to 81 bases (90 bases correspond to sequence 3) up to sequence 4 through the decrease by each one base from the 3'-side of sequence 1 also have the activity.

[0039]

The RNA aptamers of sequences 1 to 4 obtained here can be provided through transcription from synthetic DNAs or through synthesis.

Compared to the RNA of previously filed patent application (JP10-242596/1998), RNA aptamers of the present invention have stronger binding activity and are made of smaller number of RNA bases, therefore these are considered to be more profitable RNA aptamers.

These RNA aptamers can specifically inhibit the binding between Ras and Raf-1 without having any effect on the kinase activity of Ras or Raf-1. In the case of cancer cell, the control of signal transduction have the possibilities of conducting anticancer behavior since the control of signal transduction inducing proliferation or differentiation of cells cease to

function. Therefore, this RNA aptamers have the possibility of becoming a novel anticancer agent. Further, it is applicable to treatment, prevention or diagnosis of various diseases in which the signal transduction system participates.

Although a downstream signaling pathway of Ras has not been completely clarified, it is possible to elucidate the signal pathway in which Ras participates by using RNA aptamers capable of specifically inhibiting some specific routes.

[0040]

Meanwhile, an antibody bound to Raf-1 mutant which is free from a kinase activity or a kinase domain of Raf-1 has been used to study the role of Ras or Raf-1 in the cell signal transduction system (Kolch, W., et al., (1991) Nature 349, 426 - 428; Kolch, W., et al., (1996) Oncogene 13, 1305 - 1314).

The Raf-1 mutant capable of being bound to Ras without having the kinase activity not only inhibits the Ras-dependent Raf-1 activity but also blocks the wide-ranging signal transduction systems including Ras. This is because the mutant inhibits the binding of Raf-1 and also has an influence on various downstream effectors of GTP-binding Ras.

[0041]

Likewise, a monoclonal antibody bound to an epitope of a kinase domain of Raf-1 inhibits all signal transduction systems including Raf-1. This is because Raf-1 is activated not only with GTP binding Ras but also through a route having no bearing

on Ras (Kolch, W., et al., (1996) Oncogene 13, 1305 - 1314).

[0042]

From this standpoint as well, it can be said that the RNA aptamer to RBD in the present invention can specifically inhibit the binding between Ras and Raf-1 without having any effect on the kinase activity of Ras or Raf-1 by the other signaling pathways.

Further, the RNA aptamer of the present invention can be expressed within cells (Good, P. D., et al., (1997) Gene Ther. 4,45-54), and can be applied to a wide-ranging field. For example, in case of treatment for human diseases, it may be applied to treatment for inflammation (Charlton, J., et al., (1997) Chem. Biol. 4, 809-816.) and RNA treatment for pneumonia (Bless, N.M., et al., (1997)Curr. Biol. 7, 877-880.).

[0043]

Thus, the RNA aptamer of the present invention specifically block RBD of "target proteins of Ras", more preferably Raf-1. Not only it can be used in an agent for controlling intracellular signal transduction, but also it is especially suited for the field of treatment, prevention or diagnosis of various diseases in which the signal transduction system participates.

[0044]

When the nucleic acids of the present invention are used in controlling the cell signal transduction system, the nucleic acids of the present invention may directly be introduced into

desired cells. It can also be introduced into cells by being inserted into viruses or the like.

Further, it is also possible that the RNA is introduced not directly but in the form of a DNA.

[0045]

When the nucleic acid of the present invention is used as a pharmaceutical composition, it can parenterally be administered as such, or it can be administered by being inserted into viruses or various vectors in the form of a DNA. In these administration forms, the pharmaceutical composition can also be provided using a pharmaceutically acceptable carrier.

The pharmaceutical composition of the present invention is useful for treatment, prevention or diagnosis of various diseases in which the cell signal transduction system participates, especially malignant tumors and inflammatory diseases.

[0046]

[Example]

The present invention is illustrated more specifically with reference to the following Examples. However, the present invention is not limited to these Examples.

[0047]

Example 1 (Formation of the first RNA pool)

A single-stranded DNA (200 pmols, 1.2×10^{14} molecules) containing random 45 bases and having a sequence 5'-ggtaa tacga

ctcac tatag ggagt ggagg aattc atcga ggcat-3' at the 5'-terminus and a sequence 5'-catat gcctt agcga cagca agctt ctgc-3' at the 3'-terminus was subjected to PCR using 2 primers, 5'-ggtaa tacga ctcac tatag ggagt ggagg aattc atcg-3' and 5'-gcaga agctt gctgt cgcta aggc-3', and then transcribed with a T7 RNA polymerase to form a first RNA pool.

[0048]

Example 2 (Selection of RNA bound to Raf-1RBD)

3 Micromols (1,800 pmols) of the RNA pool heated at 75°C for 3 minutes and then ice-cooled and 1 μM (600 pmols) of GST-RBD were incubated in 600 μl of a binding buffer at 37°C for 1 hour. The culture was filtered with a nitrocellulose filter, and the filter was cleaned three times with 300 μl of a cleaning buffer. Thereafter, the RNAs on the filter were eluted with a buffer containing 7 M urea. After the reverse transcription, PCR was conducted at 12 cycles.

The reagents used here are as follows.

GST-RBD: A fusion protein of RBD (51 to 131 amino acid moiety of Raf-1) and glutathione-S-transferase is described in a literature (Shirouzu, M., et al., (1998) J. Biol. Chem. 273, 7737 - 7742).

binding buffer: 5 mM $MgCl_2$-containing phosphate buffer physiological saline solution

cleaning buffer: 20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$ and 150 mM NaCl

[0049]

Example 3 (RNAs of sequence Nos. 1 and 2)

Selection of RNAs bound to Raf-1 RBD from the first RNA pool in Example 2, reverse transcription of RNAs to DNAs, amplification and transcription of DNAs to RNAs were repeated 10 times to obtain RNAs of sequence Nos. 1 and 2 of Sequence Listing.

[0050]

Example 4 (RNAs of sequence Nos. 3 and 4)

DNAs which had a complementary sequence of an RNA of sequence No. 1 and of which the 3'-terminus side was shortened were obtained by PCR using a primer 5'-ggtaa tacga ctcac tatag ggaggt ggagg aattc atcg-3' and a primer 5'-gctgt cgcta aggca tatgc taaaa c-3' or 5'-aggca tatgc taaaa ccaat ttata ac-3'. From these DNAs, RNAs of sequence Nos. 3 and 4 of Sequence Listing were obtained.

[0051]

Example 5 (Cloning and determination of a sequence)

A DNA was cloned using a TOPO TA cloning kit, and the sequence was determined with an automatic DNA sequencer.

[0052]

Example 6 (Measurement of a Kd value)

An RNA (4 nM) of which the 5'-terminus was labeled and 50 to 1,250 nM of GST-RBD were incubated in 600 μl of a binding buffer at 37°C for 30 minutes. The culture was filtered with a nitrocellulose filter, and the radioactivity on the filter

was measured. The Kd value was calculated using a software: Kalleider Graph (Bell, S. D., et al., (1998) J. Biol. Chem. 273, 14309 - 14314).

[0053]

Example 7 (Binding inhibition experiment)

GST-RBD (20 pmols) in 160 μl of a binding buffer containing 0.05% Triton X-100 and 10 μl of phosphate buffer physiological saline solution containing glutathione-Sepharose 4B beads were mixed, and the mixture was incubated at 4°C for 30 minutes. The beads were separated, and incubated with 20 pmols of Ras and an RNA (0, 20, 100 and 200 pmols) in 160 μl of binding buffer (5 mM $MgCl_2$-containing phosphate buffer physiological saline solution) at 4°C for 30 minutes. After the beads were cleaned, the bound protein was eluted with a Laemmli's buffer, subjected to SDS-PAGE, then immunoblotted using anti-Ras antibody RAS004 (Kanai, T., et al., (1987) Jpn. J. Cancer Res. 78, 1314 - 1318), and visualized with an ECL immune detector.

In the RNAs of sequence Nos. 1 to 4, the decrease in the amount of Ras was observed according to the RNA amount.

[0054]

[Effects of the Invention]

The present invention provides RNA which is specifically bound to target proteins of Ras such as Raf-1 and the like and which further inhibit the binding to Ras, and a method of specifically inhibiting an intracellular signaling pathway

using these RNAs. The present invention can not only clarify the signaling pathway through the specific route of cells but also provide a pharmaceutical composition having less side effects.

The RNA aptamer of sequences 1 to 4 obtained here can be provided through transcription from synthetic DNAs or through synthesis.

Compared to the RNA of previously filed patent application, the present invention has stronger binding activity and is made of smaller number of RNA bases, therefore it is considered to be more profitable.

These RNA aptamers can specifically inhibit the binding between Ras and Raf-1 without having any effect on the kinase activity of Ras or Raf-1. In the case of cancer cell, the control of signal transduction have the possibilities of conducting anticancer behavior since the control of signal transduction inducing proliferation or differentiation of cells cease to function. Therefore, this RNA aptamer has the possibility of becoming a novel anticancer agent. Further, it is applicable to treatment, prevention or diagnosis of various diseases in which the signal transduction system participates.

[Sequence Listing]

SEQUENCE LISTING

<110> Japan Science And Technology Corporation

<120> Nucleic acid which may bind specifically to proteins Raf-1

<130> PA907855

<160> 4

<210> 1

<211> 100

<212> RNA

<213> Artificial Sequence

<220>

<221> protein bind

<223> RNA aptamer

<400> 1

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auugguuuua gcauaugccu uagcgacagc aagcuucugc 100

<210> 2

<211> 98

<212> RNA

<213> Artificial Sequence

<220>

<221> protein bind

<223> RNA aptamer

<400> 2

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ccuacacuuc ucaugccuua gcgacagcaa gcuucugc 98

<210> 3

<211> 90

<212> RNA

<213> Artificial Sequence

<220>

<221> protein bind

<223> RNA aptamer

<400> 3

gggaguggag gaauucaucg aggcauaugu cgacuccguc uuccuucaaa ccaguuauaa 60

auugguuuua gcauaugccu uagcgacagc 90

<210> 4

<211> 80

<212> RNA

<213> Artificial Sequence

<220>

<221> protein bind

<223> RNA aptamer

<400> 4

gggaguggag gaauucaucg aggcauaugu cgacuccguc uuccuucaaa ccaguuauaa 60

auugguuuua gcauaugccu 80

[Document] Abstract

[Abstract]

[Problem]

The present invention provides a novel nucleic acid in order to clarify the signaling pathway in which the target proteins of Ras participate and the physiological activity provided by the inhibition of the signal transduction, the development of substances which specifically inhibit the binding to Ras particularly having an important role in cells along with Raf-1, by being specifically bound to the target proteins of Ras, in more detail, Raf-1 and which have a strong activity.

[Means for Solving Problem]

The present invention relates to an RNA aptamer that bound to a target protein of Ras, preferably Raf-1, especially its RBD, an agent for controlling a signal transduction system using this RNA aptamer, a method of controlling the same, and a pharmaceutical composition containing the same.

[Chosen Drawing] None